Proposed synopsis on

Pharmacological Screening of Some Indian Medicinal Plants for Nootropic Activity

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Index

Sr. No.	Content	Page No.
1	Introduction	01
2	Review of literature	03
3	Objectives	07
4	Need of study	08
5	Plan of work	09
6	Material & Methods	10
7	Duration of work	12
8	Research outcome	13
9	References	14

Introduction:

Dementia is a syndrome that can be caused by a number of diseases that over time destroy nerve cells and damages the brain, typically resulting in impaired cognitive function. It is a term for several diseases that affect memory, thinking and the ability to perform daily activities[1]. As per WHO, Currently more than 55 million people have dementia worldwide, over 60% of whom live in low and middle income countries. Every year, there are nearly 10 million new cases. Dementia results from a variety of diseases and injuries that affect the brain. Alzheimer's disease is the most common form of dementia and can contribute to 60-70% of cases[2].Dementia can be of neurodegenerative origin (irreversible) or non-neurodegenerative origin (potentially reversible) [3]. Neurodegeneration, to some extent, is the main cause of dementia in the elderly. Few examples of frequent degenerative dementias among the elderly include:

- Alzheimer’s disease
- Parkinson’s disease
- Frontotemporal Lobar degeneration
- Dementia with Lewy bodies
- Vascular dementia
- Non Parkinsonian movement disorder, like Hungtinton‟s disease, Wilson disease
- Motor neuron disease (Amyotrophic lateral sclerosis, Primary Lateral sclerosis)

Alzheimer's disease (AD) is characterized by memory deficits, altered behavior and impaired cognitive function. The most important risk factor for AD is aging and the presence of excessive amounts of neurotic plaques containing amyloid β protein loss of cholinergic markers in brain [4]. The central cholinergic system play important role in process of learning and memory. Acetylcholine (Ach) is one of the brain neurotransmitters involved in learning, memory and attention processes. A decrease in acetylcholine in the brain of patients with AD appears to be a critical element in producing dementia [5]. The centrally acting anti- muscarinic drugs like scopolamine impairs learning and memory in rodents and human being. Age, stress and emotion are conditions that may lead to memory loss, amnesia, dementia, anxiety, schizophrenia and Alzheimer’s diseases [6].

The nootropics are the agents used in treatment of AD, called as nootropic drugs belong to the class of psychotropic agents. The term nootropic was coined by Giurgea in 1972, the word

nootropic is derived from two Greek words, Noon means mind and Tropos means turn, collectively known as tuning of mind [7]. Nootropics are also referred as smart drugs, improve mental function such as memory, also increase blood circulation to brain and improves oxygen supply to brain. Synthetic medicines like tacrine, dopnezil, aniracetam, piracetam and rivastigmine are used for treatment of cognitive dysfunction and memory loss associated with AD. But these drugs possess adverse effects including gastrointestinal disturbances and problems associated with their bioavailability. Various studies suggest that the plants traditionally used in Asia, China and Europe have a significant impact on aging and AD is involved in neurodegeneration pathways [8-9].

A range of traditional plants imparts effects via acetylcholinesterase activity, β-amyloid peptide formation in plaques, neurotrophic factors and through antioxidant activity. It is necessary to look for more effective treatment for AD with the minimum side effects. According to WHO, Presently 80 % of the world's population relies on traditional medicines and plant extracts and the active constituents are used to meet people's primary health care needs. Plants contain rich source of primary and secondary metabolites for improving health problems. Pharmaceutical industry is facing intriguing challenges like elevated cost and unendurable risk management due to the high burden of neurodegenerative disorders. A significant shift of drug discovery is being witnessed from synthetic moieties to herbal formulation [10].

Review of literature:

1. Aykac et al., (2019). Revealed the factors involved in the development of Alzheimer's disease (AD) and compare the effects of Galantamine treatment with the effects of Myrtus communis treatment. The study investigated the expression levels of M1, ACh, BDNF; AChE activity, GSH level, MDA and MPO activity, and AChE gene expression in a rat model of AD induced by scopolamine. The findings revealed that the administration of Myrtus communis significantly improved the scopolamine-induced cognitive impairments, as evidenced by improved latency and object recognition time. Furthermore, Myrtus communis treatment increased the expression levels of BDNF, M1, and ACh receptors in various brain regions. Additionally, Myrtus communis exhibited enhanced AChE activity by promoting GSH activity while reducing MDA levels and MPO activity. Based on these results, Myrtus communis holds promise as a potential novel therapeutic approach and may serve as an alternative strategy for the prevention and treatment of AD [11].

2.Tiwari et al., (2019). Reviewed on the leading causes of progressive dementia is Alzheimer's disease (AD), which is characterized by neurodegeneration. The development of AD has been linked to the accumulation of extracellular amyloid β (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated τ-protein in specific regions of the brain, such as the cortex and limbic system. This condition is marked by the loss of memory and gradual cognitive decline. The abnormal processing of amyloid precursor protein (APP) by enzymes known as β-secretases and γ-secretases leads to the production of Aβ40 and Aβ42 monomers, which subsequently aggregate into oligomers and form senile plaques. The progression of the disease can also be influenced by factors such as HIV infection. Additionally, during the pathogenesis of AD, the presence of elevated levels of Aβ peptides in the central nervous system triggers the infiltration of microglial cells [12].

3. Kumar A. et al., (2015). Reviewed a comprehensive understanding of the pathophysiology and progression of Alzheimer's disease (AD) while shedding light on potential therapeutic targets. Despite the wealth of knowledge surrounding this complex disease, there are only a limited number of treatment options available. Currently, the available drugs such as

acetylcholinesterase inhibitors (AChEIs) and memantine primarily address the symptoms of the disease rather than targeting its underlying cause. Therefore, there is hope for the development of novel therapies that can address the root cause of AD and halt the progressive accumulation of amyloid β (Ab). One promising approach is targeting Ab production through the inhibition of β-secretase, although only a few compounds have been tested and undergone clinical trials thus far [13].

4. Yadang et al., (2020). Investigated the effects of an aqueous extract derived from Carissa edulis on a Scopolamine-induced mouse model of memory impairment, with a focus on Alzheimer's disease-related cognitive deficits. Administration of Scopolamine resulted in a decline in learning and memory performance during the behavioral tests. The mice exhibited reduced time spent in the preferred arm of the T-maze, decreased exploration of the novel object, and a lower discrimination index for the familiar object. However, treatment with different doses of the Carissa edulis extract significantly improved memory performance in the T-maze and novel object recognition tests, and also ameliorated locomotion in the open field test. Furthermore, the extract decreased AChE activity in the brain, suggesting an enhancement of the cholinergic system. Additionally, the extract demonstrated antioxidant properties by reducing oxidative stress, as evidenced by a decrease in MDA level and an increase in glutathione level and catalase activity.

In conclusion, the administration of the aqueous extract derived from Carissa edulis improved memory performance in mice with Scopolamine-induced memory impairment. This improvement was attributed to the reduction in AChE activity and the antioxidant properties of the extract [14].

5. Wahid M. et al., (2020). Reviewed pharmacological exploration of traditional plants for the treatment of neurodegenerative disorders. Plants are the most blissful natural blessing as they contain unlimited sources of primary and secondary bioactive metabolites for improving human health. The pharmaceutical industry is facing many challenges, that is, being expensive, at higher risk, and more importantly being critically inefficient due to high burden of the neurodegenerative disorders. A significant shift in drug discovery is being witnessed from

synthetic moieties to herbal formulation. Natural products are the potential sources of others single or multiple active constitute that may be used to treat the neurological disorders [15].

6. Tuzimski T.et al., (2022). Identified new, safe and effective compounds that are potential candidates for further in vivo and clinical tests from which more effective drugs for the treatment of Alzheimer's disease could be selected. Author discussed the relevance of plant-derived compounds with in vitro activity. Various plants and phytochemical compounds have shown different activity that could be beneficial in the treatment of Alzheimer's disorders. Most often, medicinal plants and their active components have been investigated as acetylcholinesterase and/or butyrylcholinesterase activity inhibitors, modifiers of β-amyloid processing and antioxidant agents. This study also aims to highlight species with assessed efficacy, usable plant parts and the most active plant components in order to identify species and compounds of interest for further study. Future research directions are suggested and recommendations made to expand the use of medicinal plants, their formulations and plant-derived active compounds to prevent, mitigate and treat Alzheimer's disease [16].

7. Laura Blaikie et al., (2022). Reviewed experimental modeling of Alzheimer's disease for therapeutic screening. A review of the experimental modelling of the disease is crucial to understanding how the current disease models can be applied to gain useful results while also considering their limitations. Disease models include in-vitro, in-vivo, ex-vivo, and in-silico systems as well as clinical trials. These systems are important for testing potential therapeutics to advance drug development, in addition to modelling the pathology of the disease to gain a greater understanding of the cause and progression [17].

8. X. Chen et al., (2021). Investigated Neuroprotective effects of ononin against the aluminium chloride-induced Alzheimer's disease in rats. The present exploration was designed to unveil the ameliorative properties of ononin against the aluminium chloride (AlCl3)-provoked AD in animals via the suppression of oxidative stress and neuroinflammation. AD was provoked to the Sprague Dawley rats through administering orally with 0.5 ml/100 g b.wt. of AlCl3 25 days and then supplemented with the 30 mg/kg of ononin orally for 25th day to 36th day. The behavioural changes were examined using open field and Morris Water Maze test. The acetylcholine esterase (AChE) activity was studied by standard method. The status of Ab1-42,

MDA, SOD, total antioxidant capacity (TAC) was quantified using respective assay kits. The interleukin(IL)-1b and TNF-a, BDNF, PPAR-c, p38MAPK, and NF-jB/p65 status was quantified using respective assay kits. Brain histology was studied using microscope. The ononin treatment effectively modulated the AlCl3-triggered behavioural alterations in the AD animals. Ononin appreciably suppressed the AChE, Ab1-42, and MDA and improved the SOD and TAC in the brain tissues of AD animals. The status of IL-1b, TNF-a, p38MAPK, and NF-jB were suppressed and the BDNF and PPAR-c contents were elevated in the brain tissues of AD animals. The outcomes brain histology analysis proved the attenuate role of ononin. Our findings recommended that the ononin treatment could ameliorate the cognitive impairment, suppress the neuroinflammation and oxidative stress in the AD animals [18].

Objectives:

- To select the suitable Indian medicinal plants for Nootropic activity.
- To perform extraction of selected Indian medicinal plants.
- To investigate phytochemicals of extracted plants.
- To evaluate nootropic effects of selected plants extracts by using animal models.
- To rationalize the information by statistically proving its effects pre-clinically.

Need of study:

- Alzheimer's disease is one of the leading neurodegenerative disease that affects a large number of elderly (late stage AD) as well as adults (familial AD).
- It gradually impairs memory and reasoning and, eventually, the capacity to do even the most basic tasks.
- It constitutes for the pathogenesis of majority of dementia cases that affects around 55million people globally. Every year, there are nearly 10 million new cases reported.
- There are very few treatment options available for AD therapy and these targets AD primarily at amyloid and tau pathology, that are still in the drug pipeline and not yet available in market.
- Secondarily, by either inhibiting acetylcholinesterase (viz., galantamine, donepezil, rivastigmine and tacrine) or preventing the damage to cholinergic neurons by glutamate (viz., memantine).
- It is necessary to look for more effective treatment for AD with the minimum side effects both primarily as well as secondarily.

Plan of work:

a) Literature survey.

b) Selection of suitable Indian medicinal plants.

c) Extraction of selected medicinal plants.

d) Phytochemical analysis of plant extracts.

e) Pharmacological Screening for Nootropic activity

- Scopolamine Induced Amnesia Model
- $AlCl_3$ Induced Alzheimer's disease

f) Statistical interpretation

Material & Methods:

1. PreliminaryPhytochemical Investigation

Phytochemical examination of selected plants will be carried out as per the standard methods [19-20].

2. Scopolamine Induced Amnesia Model

Induction of Alzheimer's disease: Alzheimer's disease mainly induced by using Scopolamine into the Wistar Rats [17].

- **Behavioural studies:**

a. Elevated plus-maze

Rodent learning and memory will be assessed using the Elevated Plus Maze (EPM), an exteroceptive behavioral paradigm [21].

b. Morris water maze (MWM) task

The MWM task will be used to gauge rat's capacity for spatial long term memory [21].

c. Y-maze task

Y-maze task will be used to measure the spatial working through the spontaneous alternation of behaviour [21-22].

d. Measurement of acetylcholine esterase (AChE) content

The content of AChE in the brain tissues of control and treated animals will be accessed by the method of Oikarinen R. et al [23].

e. Histopathological study

Histopathology of brain cortex and dentate gyrus of rat brain will be performed.

3. $AlCl_3$ Induced Alzheimer's disease Model

Induction of Alzheimer's disease: Alzheimer's disease mainly induced by using Aluminium Chloride into the Wistar Rats [17].

- **Behavioural studies:**

a. Radial arm maze (RAM) test

The RAM test will be performed according to Foyet et al. [24].

b. **Morris water maze (MWM) task:**

The MWM task will be used to gauge rat's capacity for spatial long term memory [21].

c. Measurement of acetylcholine esterase (AChE) content

The content of AChE in the brain tissues of control and treated animals will be accessed by the method of Oikarinen R. et al [23].

d. Histopathological study

Histopathology of brain cortex and dentate gyrus of rat brain will be performed.

4. Data and statistical analysis:

Data were expressed as mean ± standard error of mean (SEM). Significance between two groups will be determined using the unpaired student's t test. Statistical comparisons between all groups will be performed by using one way ANOVA followed by the Dunnett test. P-value < 0.05 will be regarded as significant.

Duration of Work:

Sr. No.	Activity	Tentative Duration (Months)	Tentative Duration (Years)
1	Literature Survey	06	01
2	Selection and collection of suitable Indian medicinal plants	03	
3	Extraction of selected medicinal plants	03	
4	Phytochemical analysis of plant extracts	03	01
5	Pharmacological Screening for Nootropic activity	09	
6	Compilation, interpretation of data and conclusion	06	01
7	Writing of thesis	03	
8	Publishing research and submitting data	03	
9	Total	36	03

Research Outcome:

Alzheimer’s disease (AD) is the most progressive form of neurodegenerative disease, which severely impairs cognitive function. Oxidative stress is identified to contribute to the mechanisms responsible for the pathogenesis of such neurodegenerative diseases. Globally, around 50 million individuals are alleged to be diagnosed with AD, and it is expected that this rate might exceed 152 million by the year 2050. Currently, AD is the fifth leading cause of death among individuals aged 65 and older, and sixth across all ages. Most of the AD cases are reported in developing countries. In most cases, roughly 68% of elderly patients belong to India, China, and South Asian countries.Synthetic medicines like tacrine, dopnezil, aniracetam, piracetam and rivastigmine are used for treatment of cognitive dysfunction and memory loss associated with AD. But these drugs possess severe adverse effects including gastrointestinal disturbances and problems associated with their bioavailability. Presently eighty percent of the world's population relies on traditional medicines and plant extracts and the active constituents are used to meet people's primary health care needs. Plants contain rich source of primary and secondary metabolites for improving health problems. Pharmaceutical industry is facing intriguing challenges like elevated cost and unendurable risk management due to the high burden of neurodegenerative disorders.

On the basis of scientific data present study taken into consideration for to screen the herbal plants for nootropic activity.

References:

1. Kumar KA, Kumar MS, Babu AN, Tony DE. Preclinical & Pharmaceutical Research evaluation of nootropic activity of leaf extract of typhaangustata. Int J Preclin Pharm Res 2014;5(2):57–60.
2. World Health Organization. (2023, March 15). “Dementia”.
3. Julie A. Schneider, Zoe Arvanitakis, Woojeong Bang, David A. Bennett. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. Neurology Dec 2007, 69 (24) 2197-2204.
4. Kaur, K., R. Kaur, and M. Kaur. “Recent Advances in Alzheimer’s Disease: Causes and Treatment”. *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 8, no. 2, Feb. 2016, pp. 8-15.
5. Kulkarni PD, Ghaisas MM, Chivate ND, Sankpal PS. Memory enhancing activity of cissampelospariera in mice. Int J Pharm Pharm Sci 2011;3(2):206–11.
6. Pal A, Jena M, Mishra S. Nootropic Activity of Zingiber Officinale in Albino Mice : A Behavioral and Neurochemical Approach. Res J Pharm, Biol Chem Sci 2013;4(4):1129–38.
7. Giurgea C (1972). "Pharmacology of integrative activity of the brain. Attempt at nootropic concept in psychopharmacology". *ActualitesPharmacologiques* (in French). 25: 115–156.
8. Gibbs RB, Mauk R, Nelson D, Johnson DA. Donepezil treatment restores the ability ofestradiol to enhance cognitive performance in aged rats: Evidence for the cholinergic basis of the critical period hypothesis. Horm Behav 2009;56(1):73–83.
9. Mukherjee PK, Kumar V, Mal M, Houghton PJ. Acetylcholinesterase inhibitors fromplants. Phytomedicine 2007;14(4):289–300.
10. Eckert GP. Traditional used Plants against Cognitive Decline and Alzheimer Disease. Front Pharmacol. 2010 Dec 8;1:138.
11. Aykac A, Ozbeyli D, Uncu M, Ertaş B, Kılınc O, Şen A, Orun O, Sener G. Evaluation of the protective effect of Myrtus communis in scopolamine-induced Alzheimer model through cholinergic receptors. Gene. 2019 Mar 20;689:194-201.
12. Tiwari S, Atluri V, Kaushik A, Yndart A, Nair M. Alzheimer's disease: pathogenesis, diagnostics, and therapeutics. Int J Nanomedicine. 2019 Jul 19;14:5541-5554.

13. Kumar, A., Singh, A. & Ekavali A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol. Rep* **67**, 195–203 (2015).
14. Yadang FSA, Nguezeye Y, Kom CW, Betote PHD, Mamat A, Tchokouaha LRY, Taiwé GS, Agbor GA, Bum EN. Scopolamine-Induced Memory Impairment in Mice: Neuroprotective Effects of *Carissa edulis* (Forssk.) Valh (Apocynaceae) Aqueous Extract. Int J Alzheimers Dis. 2020 Aug 31;2020:6372059. doi: 10.1155/2020/6372059.
15. Wahid M, Ali A, Saqib F, Aleem A, Bibi S, Afzal K, Ali A, Baig A, Khan SA, Bin Asad MHH. Pharmacological exploration of traditional plants for the treatment of neurodegenerative disorders. Phytother Res. 2020 Dec;34(12):3089-3112.
16. Tuzimski T, Petruczynik A. Determination of Anti-Alzheimer's Disease Activity of Selected Plant Ingredients. Molecules. 2022 May 18;27(10):3222.
17. Laura Blaikie, Graeme Kay, Patricia Maciel, Paul Kong Thoo Lin,Experimental modelling of Alzheimer's disease for therapeutic screening,European Journal of Medicinal Chemistry Reports, Volume 5 (2022), 100044.
18. Chen X, Zhang M, Ahmed M, Surapaneni KM, Veeraraghavan VP, Arulselvan P. Neuroprotective effects of ononin against the aluminium chloride-induced Alzheimer's disease in rats. Saudi J Biol Sci. 2021 Aug;28(8):4232-4239.
19. Kokate CK, Purohit AP, Gokale SB. Pharmacognosy. 43rd Edition, Pune (India): Nirali Prakashan, 2009, pp. A1-A6.
20. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Pune (India): Nirali Prakashan, 2000.
21. H. G. Vogel and W. H. Vogel. Drug Discovery and Evaluation - Pharmacological Assays. Second Edition, Springer, Berlin, 2002.
22. Miedel CJ, Patton JM, Miedel AN, Miedel ES, Levenson JM (2017) Assessment of spontaneous alternation, novel object recognition and limb clasping in transgenic mouse models of amyloid-beta and tau neuropathology. J Vis Exp 123:66.
23. Oikarinen R, Molnár GK, Kalimo H, Riekkinen PJ. Cholinesterase activities in the somatic nervous system of rabbits with experimental allergic neuritis. Exp Neurol. 1983 Mar;79(3):601-10.
24. H.S. Foyet, A.E. Asongalem, E.K. Oben, O. Cioanca, M. Hancianu, L. Hritcu, Effects of the methanolic extract of Vitellaria paradoxa stem bark against scopolamineinduced

cognitive dysfunction and oxidative stress in the Rat Hippocampus, Cell. Mol. Neurobiol. 36 (7) (2016) 1139–1149.

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